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14. ABSTRACT
A new protocol was developed for laboratory study of the interaction of hazardous bioaerosols with shock/blast waves. The method determined the viability of endospores as a function of shock strength (post-shock temperature) and used laser-based diagnostics to monitor the products of endospore rupture. Aqueous aerosols laden with bacterial endospores and silica counting beads were produced and the subsequent fog was loaded into the test region of a gas-driven shock tube where it was subjected to shock waves of controlled strength. In situ optical

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Ronald Hanson
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Report Title

Final Report: Interaction of Bio-Aerosols with Shock/Blast Waves: Dispersion, Activation, and Destruction of Airborne Biological Threats

ABSTRACT

A new protocol was developed for laboratory study of the interaction of hazardous bioaerosols with shock/blast waves. The method determined the viability of endospores as a function of shock strength (post-shock temperature) and used laser-based diagnostics to monitor the products of endospore rupture. Aqueous aerosols laden with bacterial endospores and silica counting beads were produced and the subsequent fog was loaded into the test region of a gas-driven shock tube where it was subjected to shock waves of controlled strength. In situ optical diagnostics monitored the water evaporation, gas temperature, and the production of UV-absorbing lysate molecules from spore rupture. Shock-treated bioaerosol samples were enumerated by flow cytometry and viability was assessed by standard plating techniques. This new experimental laboratory protocol was used to investigate the post-shock-heating survival of three strains of endospores (Bacillus atrophaeus, Bacillus subtilis and Bacillus thuringiensis, Al Hakam). The loss of viability by shock-treated endospores was discovered to follow an exponential decay with temperature (Arrhenius rate behavior) for the three strains of endospores investigated. The in situ laser extinction measurements were used to determine the rate of spore destruction as a function of post-shock temperature.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

S.D. Gates, A.D. McCartt, P. Lappas, J.B. Jeffries, R.K. Hanson, L.A. Hokama and K.E. Mortelmans, “Bacillus endospore resistance to gasdynamic,” J. Applied Microbiology 109 (2010) 1591-1598.

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

1. P. Lappas, A.D. McCartt, C. Strand, S.D. Gates, D.F. Davidson, J.B. Jeffries, R.K. Hanson, L-M Joubert, L.A. Hokama, K.E. Mortelmans, “Dispersion, activation, and destruction of airborne biological threats: Laboratory studies of the interaction of spore-laden aerosols with shock/blast waves,” Chemical and Biological Defense Physical Science and Technology Conference, New Orleans, LA, November, 2008.
2. A.D. McCartt, S.D. Gates, P. Lappas, J.B. Jeffries, R.K. Hanson, “In Situ Optical Measurements of Shock Wave Interactions with Endospore-Laden Bioaerosols,” 2009 Chemical and Biological Defense Science and Technology Conference, Dallas, TX, November, 2009.
3. S.D. Gates, A.D. McCartt, P. Lappas, J.B. Jeffries, R.K. Hanson, L.A. Hokama, K.E. Mortelmans, “Ex Situ Analysis of Shock Wave Induced Damage to Bacillus Globigii (BG) Endospores,” 2009 Chemical and Biological Defense Science and Technology Conference, Dallas, TX, November, 2009.

Number of Papers published in non peer-reviewed journals: 3.00

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

1. S.D. Gates, A.D. McCartt, J.B. Jeffries, R.K. Hanson, L.A. Hokama, K.E. Mortelmans, “Extension of Bacillus endospore gas dynamic heating studies to multiple strains and test conditions,” J. Applied Microbiology, submitted April 2011.
3. A.D. McCartt, S.D. Gates, J.B. Jeffries, R.K. Hanson, T.L. Buhr, “Response of Bacillus thuringiensis Al Hakam endospores to gas dynamic heating in a shock tube,” Letters Applied Microbiology, submitted May 2011.
4. A.D. McCartt, S.D. Gates, P. Lappas, J.B. Jeffries, R.K. Hanson, “In-situ optical measurements of bacterial endospore breakdown in a shock tube,” Applied Physics B, submitted May 2011.

Number of Manuscripts: 3.00

Patents Submitted

Patents Awarded

Awards

R.K. Hanson Egerton Gold Medal of the Combustion Institute 2008
R.K. Hanson Hottel Lecturer for the Combustion Institute 2010

Graduate Students

NAME	PERCENT_SUPPORTED
Sean Gates	1.00
Daniel McCartt	1.00
FTE Equivalent:	**2.00**
Total Number:	**2**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
R.K. Hanson	0.08	Yes
FTE Equivalent:	**0.08**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:......	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:......	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED	
J.B. Jeffries	0.10	No
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

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H T G L

Final Technical Report

Interaction of Bio-Aerosols with Shock/Blast Waves: Dispersion, Activation, and Destruction of Airborne Biological Threats

Performance Period: 1 March 2007- 28 February 2011

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May, 2011

HIGH TEMPERATURE GASDYNAMICS LABORATORY
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Table of Contents

Interaction of Bio-Aerosols with Shock/Blast Waves: Dispersion, Activation, and Destruction of Airborne Biological Threats

by Ronald K. Hanson, PI and Professor and Jay B. Jeffries, Senior Research Engineer
Department of Mechanical Engineering Stanford University
with Kristien Mortelmans, Director of Microbiology, SRI International

Abstract

A new protocol was developed for laboratory study of the interaction of hazardous bioaerosols with shock/blast waves. The method determines the viability of endospores as a function of shock strength (post-shock temperature) and uses laser-based diagnostics to monitor the products of endospore rupture. Aqueous aerosols laden with bacterial endospores and silica counting beads were produced and the subsequent fog was loaded into the test region of a gas-driven shock tube where it was subjected to shock waves of controlled strength. *In situ* optical diagnostics monitored the water evaporation, gas temperature, and the production of UV-absorbing lysate molecules from spore rupture. Shock-treated bioaerosol samples were enumerated by flow cytometry and viability was assessed by standard plating techniques. This new experimental laboratory protocol was used to investigate the post-shock-heating survival of three strains of endospores (*Bacillus atrophaeus, Bacillus subtilis* and *Bacillus thuringiensis,* Al Hakam). The loss of viability by shock-treated endospores was discovered to follow an exponential decay with temperature (Arrhenius rate behavior) for the three strains of endospores investigated. The *in situ* laser extinction measurements were used to determine the rate of spore destruction as a function of post-shock temperature.

Project Goals

Detailed understanding of the interactions of shock/blast waves with hazardous bio-aerosols is crucial for the intelligent design of concepts for protection and/or cleanup of airborne threats. Toxic aerosols can be used for a wide variety of airborne threats including toxic agents (e.g., incapacitating and lethal chemicals) and biological agents (e.g., incapacitating and lethal disease-producing bacteria in spore and/or vegetative form, and viruses). Interactions of these aerosols with shock/blast waves have the potential to remediate or exacerbate the threat to security. The fate of hazardous aerosols after the passage of shock/blast waves depends on a multitude of complex interactions, which have the potential to promote or eliminate the threat. For example:

1) Large liquid aerosol droplets may break-up into smaller ones depending on shock strength and various gas and aerosol properties, thereby modifying settling times and potential human risk factors.
2) Aerosol particles may be dispersed with the blast cloud into a larger volume.
3) Shock heating may induce evaporation and/or chemical (or thermal) decomposition.
4) Shock/blast waves may be attenuated by passage through aerosol clouds.
5) Shock heating and rapid mechanical acceleration and stress (i.e., pressure) changes can alter the viability of biological agents.

The development of strategies for laboratory investigations of the interaction of shock/blast waves with bacterial spores is the primary goal of this project. The viability of endospores in an

aqueous aerosol was investigated as a function of shock strength, e.g. to understand the pressure/temperature/velocity that leads to destruction of the spore by chemical, thermal or mechanical forces.

Accomplishments

During the course of this project a new laboratory protocol was developed to study the interaction of endospores with shock/blast waves as illustrated in Fig. 1. A shock tube was modified to accept endospore-laden aqueous aerosols in the test gas. The test gas was then subjected to shock/blast waves of controlled strength and the effects of this shock-heating were investigated by a combination of *in situ* and *ex situ* diagnostics. The *in situ* measurements monitored multi-wavelength optical extinction and provided time-resolved measurements of the morphological destruction of the spores. Sampled of the shock-treated spores were collected for *ex situ* examination; scanning electron microscopy and flow cytometry were used to determine post-shock morphological changes in the endospores, and a standard plating technique was used to determine viability of the shock-heated endospores.

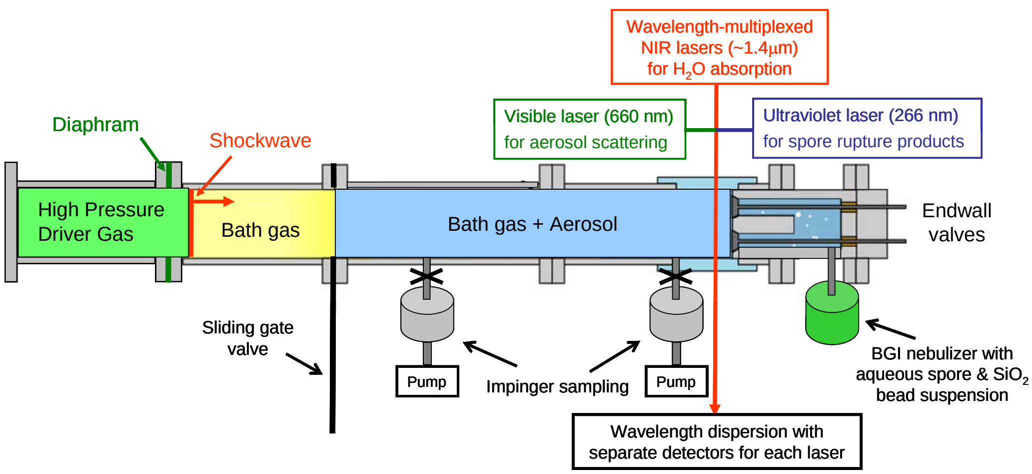

Figure 1: Schematic of the driven section of the Stanford aerosol shock tube (AST) facility configured for shock-heating spore-laden aerosol. Three *in situ* laser diagnostics, 5 cm from the endwall, monitor the shock-heated gas/aerosol, while pre- and post-shock samples are taken with gas dynamic impingers for *ex situ* analysis. (Not drawn to scale).

Key accomplishments of this project include (1) the development and demonstration of the first laboratory method for study of the interaction of endospores with shock waves of controlled strength, (2) the development and demonstration of a laser-extinction scheme to monitor spore time-resolved rates of endospore destruction, and (3) the investigation of the loss of viability as a function of shock heating for three strains of endospores.

Three different strains of endospores were investigated: *Bacillus subtilis*, *Bacillus atrophaeus* and *Bacillus thuringiensis* (Al Hakam). The exposporium on these three strains are radically different, ranging from a thin, smooth protein coat (*Bacillus subtilis*) to a complex, thick, sticky shell (*Bacillus thuringiensis*, Al Hakam). However, all three strains exhibited quite similar behavior as a function of post-shock temperature shown in Fig. 2 for viability and in Fig. 3 for the destruction of the endospores structure. The endospores treated with weak shocks (post-shock temperature (T_5)<500K) retained significant viability and experienced little to no morphological degradation. As the shock strength was increased with T_5 between roughly 480

and 750 K, the endospores exhibited a rapid rate of decline in viability and a corresponding but significantly slower rate of severe spore morphological breakdown coupled with the release of lysate. For post-shock temperatures greater than 750 K, very low or even no post-shock viability was observed and the majority of spores experienced complete morphological deterioration and the substantial release of lysate.

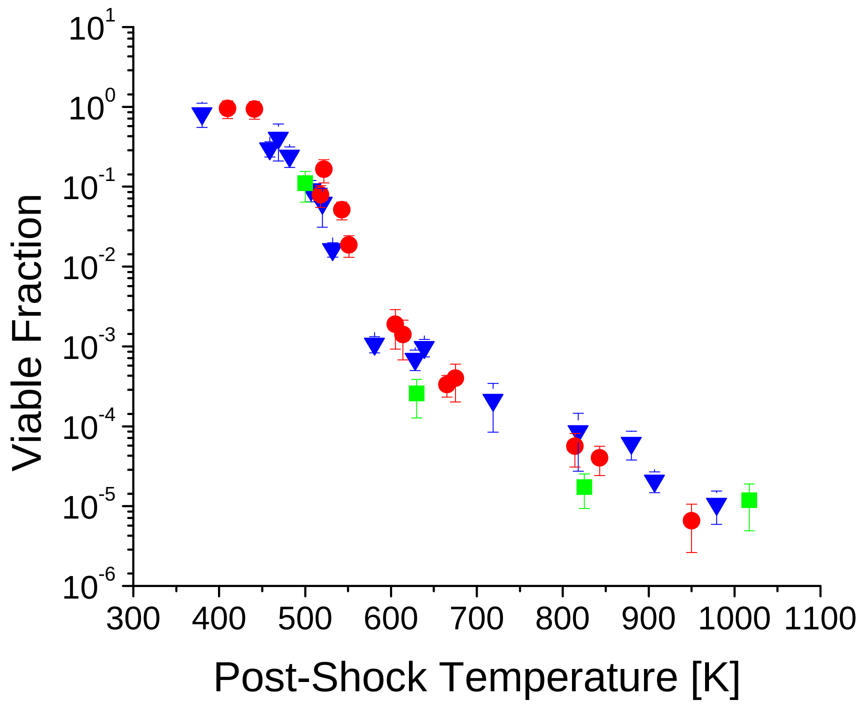

Figure 2: Viable fraction of shock-treated endospores plotted versus post-reflected shock temperature (T_5) for a test time of 2.5 ms. Triangle: *B.atrophaeus*. Circle: *B. subtilis*. Square: *B. thuringiensis* Al Hakam.

Recommendations for Future Research

We postulate that the aerosol shock tube is an ideal test bed to investigate the sensitivity of endospores survival to a combination of blast waves and chemical additives. The current work uses argon, oxygen, and air as bath gas, representing shock treatment of endospores in clean air. The next stages would mix different additives with spores. Here we hypothesize that some additives would make endospores such as the *Bacillus anthracis* simulatent *Bacillus thuringiensis* Al Hakam much more resistant to shock heating than others. We also hypothesize that such additives will have different impacts on different spore strains.

At this point we have characterized spore lysates of relatively clean spores using our UV laser absorption diagnostics. Characterization of the spore quality used in the the experiment becomes extremely critical in the next stage. Here we would suggest collaboration with researchers at the

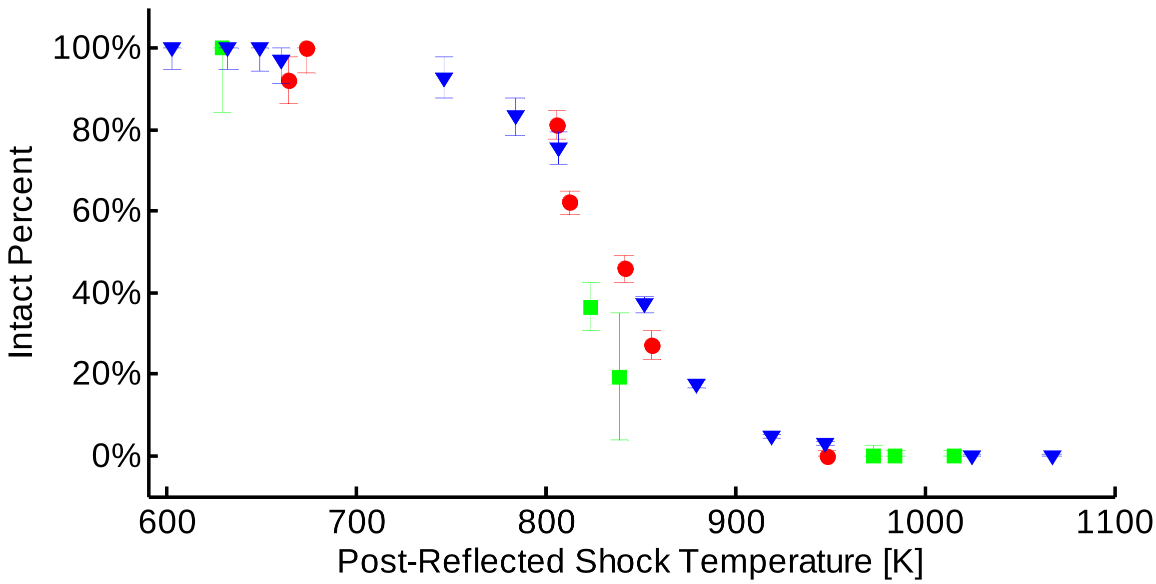

Figure 3: Percentage of endspores morphology that remain intact after the high temperature region 5 test time plotted vs post-reflected shock temperature (T_5). Triangle: *B.atrophaeus*. Circle: *B. subtilis*. Square: *B. thuringiensis* Al Hakam.

government laboratories (e.g., Dr. Buhr at the Naval Weapons Lab), to establish high-purity benchmark spores for testing.

We also suggest that a series of aerosol shock tube studies can provide the database to build and validate a predictive mechanical model to describe endospore response to shock-heated flows. More specifically, we suggest development of a theory that will describe the rate of viability loss and morphological deterioration from a nano-mechanics point of view. Experiments pertaining to this research area include long test-time measurements, which utilize tailored driver gases to achieve test times varying from 3 ms to 45 ms in the aerosol shock tube. By examining the viability curves from such tests we could potentially infer rates of heat transfer through the coat and other proteinaceous layers. Such studies could provide valuable information regarding the physical properties of the endospore coat. Using the results of these tests we would have the potential to formulate a mechanical model of the spore incorporating effects of heat transfer and mechanical stresses.

Another direction of study would be to conduct tests utilizing mutant spores lacking specific structural proteins, to enable identification of critical biological components that contribute to the resistance of endospores viability to shock heating. Here we suggest the use of a strain whose genome has been sequenced such as *Bacillus subtilis*. Such knowledge of the sequence enables the preparation of genetically modified endospores lacking critical proteins and absorbing lysate molecules. Utilizing an antibiotic marker, specific protein and dipicolinic acid DNA sequences could be modified or eliminated resulting in the production of spores with specific mutations. These modified strains could then be shock treated in our aerosol shock tube to attempt to identify proteins that are critical to resistance to shock heating. Such research would also more accurately determine the specific components of the absorbing lysate allowing optimization of the realtime optical diagnostic for endospores destruction.

Publications

All of the results and details of the research performed on this grant have been published or submitted for publication in the refereed literature

1. S.D. Gates, A.D. McCartt, P. Lappas, J.B. Jeffries, R.K. Hanson, L.A. Hokama and K.E. Mortelmans, "Bacillus endospore resistance to gasdynamic," *J. Applied Microbiology* **109** (2010) 1591-1598. This paper describes the aerosol shock tube, the protocol and proceedures for the study of the interaction of shockwaves with endospore-laden aersols. Measurements on *Bacillus atrophaeus* in an argon bath gas were used to demonstrate this experimental method.
2. S.D. Gates, A.D. McCartt, J.B. Jeffries, R.K. Hanson, L.A. Hokama, K.E. Mortelmans, "Extension of *Bacillus* endospore gas dynamic heating studies to multiple strains and test conditions," *J. Applied Microbiology*, submitted April 2011. This paper extends the investigation of the resistance of endospores viability to shock heating to *Bacillus subtilis*. In addition, the use of bath gas with and without oxygen was investigated to understand any potential role of oxidation in the destruction of endospores.
3. A.D. McCartt, S.D. Gates, J.B. Jeffries, R.K. Hanson, T.L. Buhr, "Response of *Bacillus thuringiensis* Al Hakam endospores to gas dynamic heating in a shock tube," *Letters Applied Microbiology*, submitted May 2011. This paper extends the investigation of the resistance of endospores viability to shock heating to *Bacillus thuringiensis* Al Hakam. This strain is a good simulant of *Bacillus anthracis* due to its complex and heavy exporium.
4. A.D. McCartt, S.D. Gates, P. Lappas, J.B. Jeffries, R.K. Hanson, "*In-situ* optical measurements of bacterial endospore breakdown in a shock tube," *Applied Physics B*, submitted May 2011. This paper describes the details of the laser extinction diagnostic to monitor time-resolved endospore destruction. These measurements offer the first ever measurements of the rate of endospore desctruction as a function of post-shock temperature. The diagnostic relies on ultraviolet (UV) absorption by the lystate. The spores are simultaneously monitored by Mie scattering at a non-absorbing wavelength.